Global Change Biology (2008) **14**, 539–555, doi: 10.1111/j.1365-2486.2007.01507.x

Carbon dioxide exchange above a Mediterranean C3/C4 grassland during two climatologically contrasting years

LUIS MIGUEL IGREJA AIRES*, CASIMIRO ADRIÃO PIO* and JOÃO SANTOS PEREIRA†
*CESAM & Departamento de Ambiente e Ordenamento, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal,
†Departamento de Engenharia Florestal, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal

Abstract

Eddy-covariance measurements of net ecosystem carbon exchange (NEE) were carried out above a grazed Mediterranean C3/C4 grassland in southern Portugal, during two hydrological years, 2004–2005 and 2005–2006, of contrasting rainfall. Here, we examine the seasonal and interannual variation in NEE and its major components, gross primary production (GPP) and ecosystem respiration (R_{eco}), in terms of the relevant biophysical controls. The first hydrological year was dry, with total precipitation 45% below the long-term mean (669 mm) and the second was normal, with total precipitation only 12% above the long-term mean. The drought conditions during the winter and early spring of the dry year limited grass production and the leaf area index (LAI) was very low. Hence, during the peak of the growth period, the maximum daily rate of NEE and the light-use and water-use efficiencies were approximately half of those observed in the normal year. In the summer of 2006, the warm-season C4 grass, *Cynodon dactylon* L., exerted an evident positive effect on NEE by converting the ecosystem into a carbon sink after strong rain events and extending the carbon sequestration for several days, after the end of senescence of the C3 grasses. On an annual basis, the GPP and NEE were 524 and 49 g C m^{-2}, respectively, for the dry year, and 1261 and −190 g C m^{-2} for the normal year. Therefore, the grassland was a moderate net source of carbon to the atmosphere, in the dry year, and a considerable net carbon sink, in the normal year. In these 2 years of experiment the total amount of precipitation was the main factor determining the interannual variation in NEE. In terms of relevant controls, GPP and NEE were strongly related to incident photosynthetic photon flux density on short-term time scales. Changes in LAI explained 84% and 77% of the variation found in GPP and NEE, respectively. Variations in R_{eco} were mainly controlled by canopy photosynthesis. After each grazing event, the reduction in LAI affected negatively the NEE.

Keywords: drought, ecosystem respiration, eddy covariance, grazing, gross primary production, harvest, light-use efficiency, Mediterranean grassland, net ecosystem carbon exchange, water-use efficiency

Received 23 April 2007; revised version received 24 September 2007 and accepted 23 October 2007

Introduction

Mediterranean climate is one of the most distinctive climates of the world, with mild winters and hot summers. Rainfall occurs normally in autumn, winter and early spring. A long dry period occurs during the warm season (summer). Furthermore, large interannual variability in the amount and pattern of rain has been observed (Miranda *et al.*, 2002). This is important because the timing of rainfall, the extent of the dry season and the regime of rain pulses determine resource availability and species composition and productivity (Schwinning & Ehleringer, 2001).

The interannual variability in total precipitation can lead to significant changes in species composition and abundance (Figueroa & Davy, 1991), grass production and net ecosystem carbon exchange (NEE) (Meyers, 2001; Sims & Bradford, 2001; Flanagan *et al.*, 2002; Suyker *et al.*, 2003; Nagy *et al.*, 2007). In a study conducted over a pasture in Oklahoma it was shown that the ecosystem switched from a net sink, in non-

Correspondence: Luis Miguel Igreja Aires, tel. + 351 234 370200, fax + 351 234 370309, e-mail: laires@dao.ua.pt

drought years, to a net source of carbon, in a drought year (Meyers, 2001). The total amount of precipitation was also the most important factor in controlling the interannual variability in the carbon exchange of a northern temperate grassland, which lost carbon during a year with precipitation below normal (Flanagan *et al*., 2002). However, some regions are characterized by the strong seasonality of water availability. In such ecosystems, carbon assimilation and productivity are often closely related with the length of the dry season (House & Hall, 2001) or the timing of the rain events (Hunt *et al*., 2004; Xu & Baldocchi, 2004). For example, from 2-year measurements of carbon dioxide (CO_2) fluxes over a Mediterranean annual grassland, Xu & Baldocchi (2004) found that the timing of rain events exerted a strong control on both the length of the growing season and the ecosystem respiration. This led the ecosystem to lose carbon in the season with slightly higher total precipitation.

Mediterranean grasslands are typically dominated by annual C3 grasses and legumes that are active during the wet period of the year. Usually their life cycle is terminated by the beginning of the dry season. Measurements of CO_2 exchange over Mediterranean grasslands were primarily conducted by Valentini *et al*. (1995), but not on a continuous basis. Until now, only Xu & Baldocchi (2004) have reported long-term measurements over a Mediterranean annual grassland with a C3 plant community. However, in some cases nonnative C4 plants invaded these communities. Although this may become more common in the future with milder winters due to climate change, there is a lack of information on how a mixture of C3/C4 species influences the carbon exchange in Mediterranean climates, especially in summer when the warm-season C4 grasses remain active.

In recent years, in Portugal, most of the annual precipitation occurs in autumn, with a negative anomaly in late winter and spring. These anomalies may become more frequent and severe as climate change scenarios for Portugal suggest a shorter and wetter rainy season, followed by a long drought period (Miranda *et al*., 2002). Therefore, understanding how climate variability, particularly reductions in precipitation and shifts its seasonality, influences the CO_2 exchange in Mediterranean grasslands, can be valuable, not only to improve our knowledge on the mechanisms that control the CO_2 fluxes, but also to anticipate possible impacts of the climate change scenarios and give the modellers a better basis to improve and validate their models.

Here, we report measurements of CO_2 fluxes during two climatologically contrasting hydrological years, one dry, 2004–2005, and another normal, 2005–2006. The hydrological year (i.e. the period from 1 October to 30 September of the next year) corresponds approximately to the annual cycle of the vegetation. The 2004–2005 hydrological year extended from Day Of the Year (DOY) 275 of 2004 to DOY 273 of 2005 and the 2005–2006 hydrological year from DOY 274 of 2005 to DOY 273 of 2006. The objectives of this investigation were to (1) examine and quantify the seasonal and interannual variation in NEE and its major components, gross primary production (GPP) and ecosystem respiration (R_{eco}), (2) examine the seasonal and interannual variation in light-use and water-use efficiencies and (3) assess the response of NEE, GPP and R_{eco} to changes in the most relevant biophysical factors.

Materials and methods

Site description

The study area was established in June of 2004 as a part of the Carboeurope IP project on a 50 ha homogeneous semi-natural grassland located in *Monte do Tojal*, Évora, in Southern Portugal (38°28′28″N; 8°01′25″W; 190 m a.s.l.). The area is fairly flat, with a minimum and maximum fetch distance of 250 and 500 m, respectively, to the flux tower. The soil is a Luvisol (FAO), containing 20% clay, 71% sand and 9% silt, and overlays a fractured granodiorite. The soil profile is about 90 cm deep and includes a relatively dense layer of clay between 25 and 50 cm depth. The average organic carbon content of this soil is 6.04 kg m^{-2}. The bulk density of the upper 30 cm of the soil profile is around 1.64 ± 0.08 g cm^{-3} ($n = 16$).

The climate is Mediterranean with hot summers and mild winters. Long-term (1951–1980) mean annual air temperature and mean annual precipitation are, respectively, 15.5 °C and 669 mm (INMG, 1991). Most of the precipitation is confined to the period between October and May.

The vegetation at the site consists of a mixture of cold-season annual (C3) grasses and one warm-season perennial (C4) grass, *Cynodon dactylon* (L.) Pers. It was grazed by sheep from late October to early February, in the first hydrological year, and from late October to late December, in the second, one or two times per week with a stocking density of 60 animals ha^{-1}. The C3 grassland community was dominated by *Avena barbata* Link ssp. *Lusitanica* (Tab. Mor.) Romero Zarco, *Vulpia bromoides* (L.) S.F. Grey, *Vulpia geniculata* (L.) Link, *Medicago sativa* L., *Medicago polymorpha* L., *Trifolium resupinatum* L., *Trifolium subterraneum* L., *Ornithopus compressus* L., *Chamaemelum mixtum* (L.) All., *Parentucellia viscosa* (L.) Caruel and *Crepis vesicaria* L. The C3 grassland community began to grow with the first rain events in autumn and senesced by about mid spring. Shoots of the C4 grass began to grow in late winter, and died in early autumn.

Field measurements

The fluxes of CO_2 were continuously measured using an eddy covariance system at a height of 2.5 m. The system consisted of a 3D sonic anemometer (model 1210R3, Gill Instruments Ltd, Lymington, UK) and an open-path infrared gas analyzer (IRGA, model LI-7500, LI-COR Inc., Lincoln, NE, USA) to measure the three components of the wind velocity, the sonic temperature and the concentrations of water vapour and CO_2, respectively. The IRGA was placed with a 30° tilt angle to minimize accumulation of dust and water droplets on the windows. The IRGA was calibrated once a month with nitrogen gas and a 350 ppm CO_2 standard, to calibrate the CO_2 and water vapour zeros and the span of CO_2, respectively. The span of water vapour was calibrated from the dew point value estimated from ambient air temperature and relative humidity, using conventional sensors and physical formulations. Real-time data were acquired at a 20 Hz sampling rate, using the software EDDYMEAS (Meteotools, Jena, Germany) and stored on a laptop computer for later processing.

Continuous measurements also included standard climatological and soil parameters. Air temperature and relative humidity were measured at the height of 1.5 m with a temperature/humidity probe (MP300, Campbell Scientific Ltd, Shepshed, UK). Photosynthetic photon flux density (PPFD) was measured at 1.5 m above the ground with a quantum sensor (LI-190SA, LI-COR, Lincoln, NE, USA). The all-wave radiation components, incoming and outgoing longwave radiation (CG-3, Kipp & Zonen, Delft, the Netherlands), incoming shortwave radiation (Casella solarimeter, Casella London Ltd, Kempston, UK) and outgoing shortwave radiation (Star-pyranometer 8101, Philipp Schenk, Wien, Australia) were measured at the height of 1.5 m. Longwave radiation was only measured from January 2005 onwards. Soil temperature was measured by Platinum Resistance Thermometers placed at the depths of 2, 10 and 20 cm. One soil heat flux plate (HFT-3, Campbell Scientific Ltd, Shepshed, UK) was buried at the depth of 8 cm and the soil heat storage above the plate was added to the final calculation of the soil heat flux. Soil volumetric water content at depths of 2, 15 and 30 cm was measured using frequency domain reflectometer probes (ML2x, Delta-T Devices, Burwell, Cambridge, UK). Precipitation was recorded by a tipping bucket rain gauge (ARG100, Environmental Measurements Ltd, Gateshead, UK). The output signals of the equipment above were scanned by a data-logger (DT-605, Datataker Ltd, Cambridge, UK) and the 30-min averages were subsequently sent and stored on a laptop computer.

Leaf area index (LAI) was determined at about 1-month intervals from April 2005 to September 2006. However, during the period of fast plant growth the sampling frequency was increased to about twice per month. On each sampling date, six patches of 0.063 m^2 areas were harvested and the green leaves were removed from the stems. In the laboratory, the green leaves were scanned using a common scanner and the area was determined by proper software (SIGMASCAN, Systat Software UK Ltd, London, UK).

Aboveground biomass was determined using generally the same material that had been harvested for the LAI determination. The green plant parts were separated from the dead plant material and their biomass was determined gravimetrically after the samples had been dried for 72 h at 65 °C. The total aboveground biomass and the percentage of dead material were hence calculated. To determine the effects of grazing on the canopy development, measurements of LAI and aboveground biomass were also performed, during the 2005–2006 hydrological year, inside six exclosure cages, of 1 m^2 each, installed around the sampling station.

Data processing and flux computation

The raw data from the eddy covariance measurements were processed off-line using the software EDDYFLUX (Meteotools, Jena, Germany). The half-hourly fluxes of CO_2 (NEE) were determined by the eddy covariance method as the mean covariance between fluctuations in vertical wind speed (w') and the CO_2 concentration (c') as follows (e.g. Fuehrer & Friehe, 2002):

$$\mathrm{NEE} \approx \overline{w'c'}. \quad (1)$$

The overbar denotes the time average. By convention, negative NEE values indicate net carbon gain by the ecosystem. The calculations included a 2D coordinate rotation, spikes detection and removal similar to Vickers & Mahrt (1997) and check for instantaneous records exceeding realistic absolute limits. In addition, the air density fluctuations were taken into account to correct the fluxes of CO_2 (Webb *et al*., 1980). In this study, we did not add the CO_2 storage term to the NEE estimations because CO_2 profile measurements were not conducted. However, after calculating the storage term, based on data from the only single point of measurement, we verified that it is rather small; adding this term to the NEE we found, after gap-filling, a change of −0.4 and 6 g C m^{-2} in the annual NEE of the first and second hydrological years, respectively.

Data quality control, gap-filling and flux partitioning

The eddy covariance technique has been found to underestimate night-time NEE, under low-atmospheric turbulence conditions (Hollinger *et al*., 1999; Anthoni

et al., 2004; Scott et al., 2004; Wohlfahrt et al., 2005). Here, we followed the current practice of plotting the night-time NEE as a function of the friction velocity (u_*). Using an approach similar to Anthoni et al. (2004), for periods with little variation in LAI and soil moisture, we found a u_* threshold of $0.08\,m\,s^{-1}$ (i.e. below which night-time NEE is dependent of u_*). Thus, the original NEE dataset was filtered in order to exclude half-hourly fluxes when u_* was $<0.08\,m\,s^{-1}$.

Subsequently, two more filters were applied to remove half-hourly fluxes that resulted from malfunction of the sensors. First, using an approach similar to Rogiers et al. (2005), the fluxes of CO_2 were discharged whenever the measured H_2O concentration differed by more than 30% from that estimated from relative humidity data, using conventional physical formulations. Those cases were related to periods when rain, dew or dust caused relevant interferences on the optical path of the open-path analyzer. Hence, the CO_2 concentrations were also affected. Second, the fluxes were also excluded if the removed spikes, or the absolute limits violations, exceeded 1% of the total records of any of the three components of wind velocity and/or CO_2 concentration.

After this filtering process, the remaining dataset was submitted to data quality tests, the integral turbulence characteristics and stationarity tests (Foken & Wichura, 1996). Whenever the mean covariance of six intervals of a time series deviated by more than 50% of the value of the covariance for the full period, the mean flux was considered nonstationary and hence excluded from the analysis. The integral characteristics of the vertical wind (σ_w/u_*) were assessed to test the development of turbulent conditions. Thus, if the measured value deviated by more than 50% of the modelled result, the turbulence was not considered well developed and the mean flux was removed. After these quality tests the remaining data were classified as 'good quality data' to submit to gap-filling and flux-partitioning procedures. We also applied the quality tests for 30% of difference allowed and, hence, the remaining data were classified as 'Highest quality data' to use in fundamental research in this study. Total data gaps during the whole study period, due to missing and rejected data, were about 42% (of which around 60% occurred during the nocturnal periods).

In this study, we examined the energy balance closure which has been considered an independent method to assess the reliability of the eddy covariance measurements (Wilson et al., 2002). For short vegetation, the energy balance closure can be written as (e.g. Kato et al., 2004; Li et al., 2006)

$$H + \lambda E \approx R_n - G, \qquad (2)$$

where H is the sensible heat flux, λE the latent heat flux, R_n the net radiation and G the soil heat flux. After performing the linear regression between the eddy fluxes ($H + \lambda E$) and the available energy ($R_n - G$), using half-hourly values, the intercept, slope and coefficient of determination (r^2) for the year 2005 were $11.47\,W\,m^{-2}$, 0.81 and 0.95, respectively. A slight degradation (1%) in the energy balance closure was observed during 2006. These results suggest that the eddy covariance measurements underestimated $H + \lambda E$ by 19–20%. Although the energy balance closure is not perfect, it is within the normal range found in most studies. Several reasons have been put forward to explain the energy imbalance, usually related to the measurements of the dependent and/or independent variables (Twine et al., 2000; Wilson et al., 2002). Giving the difficulty to identify and quantify all sources of the energy imbalance, we will not advance speculative explanations. However, using daily values (where G is close to zero) in the regression for the whole study period, the energy imbalance decreased to 12%. Also, the energy balance ratio (EBR) (Wilson et al., 2002) for the whole study period indicated a lesser underestimation (EBR = 0.87).

To evaluate the footprint of flux measurements and the contribution of the study area to the total flux measured by the eddy covariance system, the forward Lagrangian stochastic trajectory model by Rannik et al. (2003) was used. The approach for site evaluation is described in Gockede et al. (2006). Even using a conservative vegetation height (0.05 m) as input, the results showed that for all atmospheric stability conditions the measurements were totally representative of the study area.

To provide complete data sets of NEE, gaps were filled following the methodology proposed by Reichstein et al. (2005). Gaps in the incoming components of radiation, temperature and precipitation data were filled with data from a nearby meteorological station. The partitioning of NEE into GPP and R_{eco} was performed according to Reichstein et al. (2005).

Data analysis

The relationship between NEE ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$) and the PPFD ($\mu mol\,m^{-2}\,s^{-1}$) was assessed by using the Michaelis–Menten rectangular hyperbola fitted to the highest quality half-hourly data, as follows (e.g. Hollinger et al., 1999; Kowalski et al., 2003):

$$NEE = \frac{GPP_{max} PPFD}{K + PPFD} + R_{eco}, \qquad (3)$$

where GPP_{max} is the gross primary productivity at infinite light ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$) and K the level of PPFD at which NEE is one half of GPP_{max}. The apparent

quantum yield (α) was determined by the ratio GPP_{max}/K. The NEE at infinite light (NEE_{max}) was derived from Eqn (3) as the sum of GPP_{max} (negative value) with R_{eco} ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$).

The relationship between night-time R_{eco}, or $NEE_{night\text{-}time}$ ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$), and the soil temperature at 2 cm depth (T_s, °C) was examined by using the *Vant Hoff* equation fitted to the highest quality half-hourly night-time data, as follows (e.g. Falge *et al*., 2001; Xu & Baldocchi, 2004):

$$NEE_{night\text{-}time} = a\exp(bT_s), \quad (4)$$

where a and b are the regression parameters. The temperature sensitivity coefficient (Q_{10}) was determined by the following equation:

$$Q_{10} = \exp(10b). \quad (5)$$

Water-use efficiency (WUE_{GPP}, $mmol\,CO_2\,mol^{-1}\,H_2O$) was calculated, on a daily basis, as the ratio between the daily-integrated GPP and the daily-integrated evapotranspiration (e.g. Reichstein *et al*., 2002b). The ecological light-use efficiency (LUE_{GPP}, $mmol\,CO_2\,mol^{-1}$ quanta) was determined, on a daily basis, as the ratio of daily-integrated GPP to daily-integrated incident PPFD (e.g. Gilmanov *et al*., 2007). To examine the seasonal variation of both WUE_{GPP} and LUE_{GPP}, 5-day running averages were used.

Results and discussion

Meteorology, LAI and aboveground biomass

Figure 1 shows the variation of the major meteorological conditions and plant parameters during the two hydrological years. The seasonal pattern of the daily-integrated shortwave radiation (R_s) was similar in the two hydrological years, with minimum values (1–5 $MJ\,m^{-2}\,day^{-1}$) in cloudy winter days and maximum values (around 30 $MJ\,m^{-2}\,day^{-1}$) in the summer (Fig. 1a–c). The number of cloudy days was substantially lower in 2005, especially between DOY 1 and 60. Maximum air temperatures (T_{max}) varied from 10 °C to 15 °C, in the winter, to extreme values (above 35 °C), during the summer (Fig. 1d–f). Minimum air temperatures (T_{min}) ranged from values around −5 °C, in winter, to values well above 10 °C, in the summer. The mean air temperature for the first and second hydrological years was 14.7 and 14.5 °C, respectively.

Precipitation was the environmental factor that differed markedly between the two hydrological years, in both the amount and pattern of rain (Fig. 1g–i). The first hydrological year was dry, with 364 mm of total precipitation (45% below the long-term mean) and the second was normal, with 751 mm of total precipitation (only 12% above the long-term mean). During the dry hydrological year, 2004–2005, there was a long period, from DOY 340 to 80, without significant rainfall (Fig. 1g and h). This led to severe plant water deficits by late winter as soil moisture content dropped below 10% in the upper 15 cm soil layer (where the C3 plants roots are distributed). In contrast, precipitation was uniformly distributed during both the winter and early spring periods of the second hydrological year, leading to high soil moisture (Fig. 1h and i). A large rain pulse occurred in late spring of 2006. Combined with a subsequent rain pulse during the summer (Fig. 1i), led to consistently higher soil moisture levels than in previous summer. It is important to mention that the higher soil moisture levels of the upper 30 cm soil layer (Fig. 1g–i), especially during the water-stressed periods, showed the capability of this soil to hold water at the depth of 30 cm, where a relatively dense layer of clay is present.

The LAI of the grazed area at the peak growth period averaged 0.4 and 2.5 in the first and second hydrological years, respectively (Fig. 1j–l). Although measurements of LAI were not performed before April 2005, LAI probably did not reach values above 1 because of low soil moisture in combination with winter grazing. The maximum LAI of the dry year laid within a range of values published for semiarid grasslands (e.g. Li *et al*., 2005), whereas for the normal year the maximum LAI was similar to those observed in an Mediterranean annual grassland in California (Xu & Baldocchi, 2004). The maximum standing biomass of the grazed area also differed markedly in the 2 years: 0.157 kg dry matter m^{-2} in the first hydrological year and 0.512 kg dry matter m^{-2} in the second (Fig. 1m–o).

In the second hydrological year, 2005–2006, grazing occurred from late October until the end of December, one or two times per week with a stocking density of 60 sheep ha^{-1}. The negative effects of grazing on LAI and aboveground biomass were well evident during winter, when low temperatures limited the growth (Fig. 1l and o). The difference in LAI and aboveground biomass between nongrazed and grazed areas was around 0.8 and 0.06 kg m^{-2}, respectively, during the winter. However, during the peak of the growth period, in early spring, the difference between nongrazed and grazed areas was substantially reduced; the LAI and aboveground biomass in the nongrazed area were, respectively, 0.4 and 0.04 kg m^{-2} higher than in the grazed area. Consistent with these results, Chocarro *et al*. (2005), from a 3-year experiment over a Mediterranean Lucerne field, found that one severe winter grazing event with sheep resulted in only a limited reduction in yield in the spring.

In both hydrological years, C3 grass species senesced by about the beginning of May (DOY 132). Thereafter,

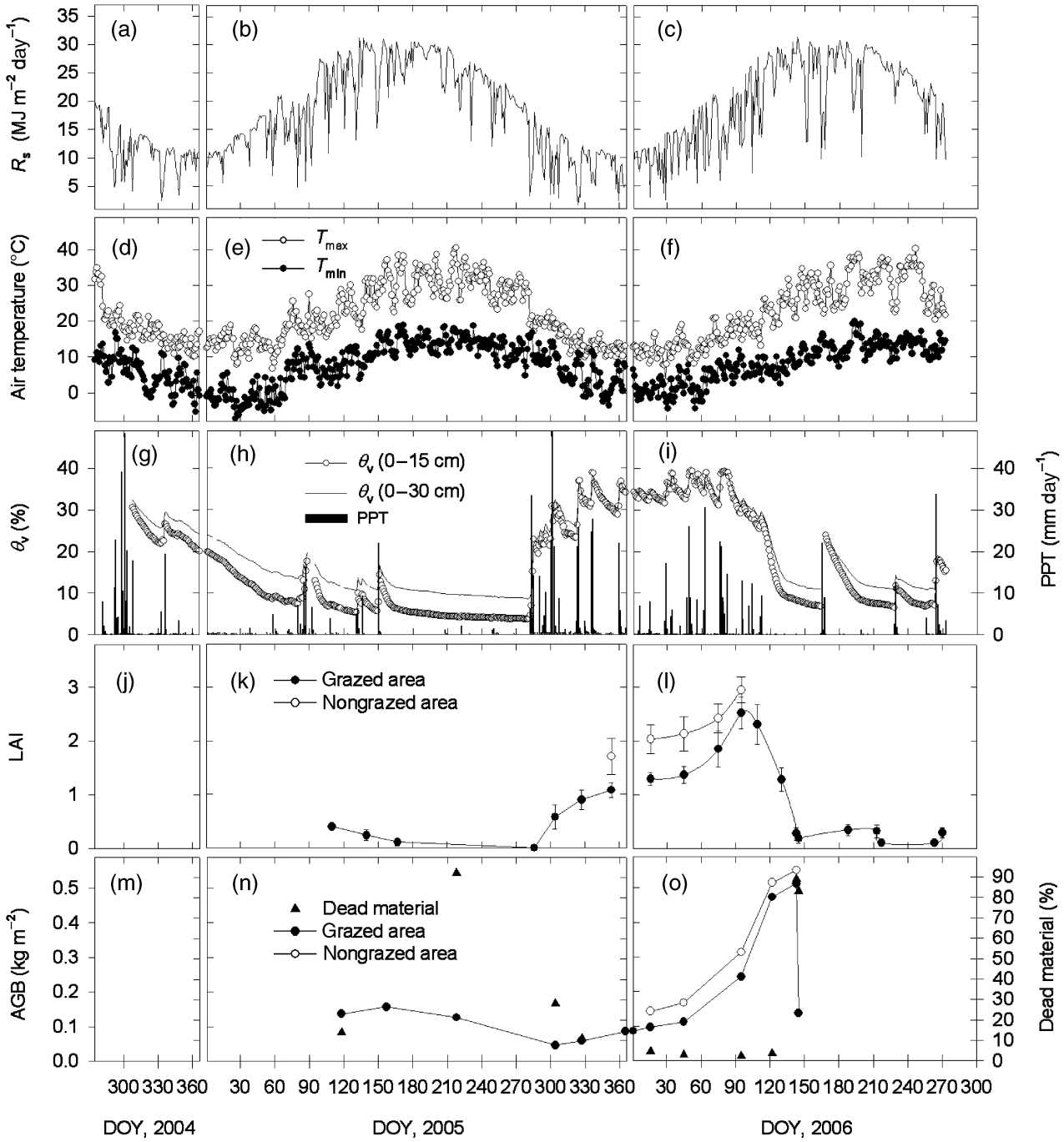


Fig. 1 Seasonal variation in (a–c) daily-integrated shortwave radiation (R_s), (d–f) maximum (T_{max}) and minimum (T_{min}) air temperature, (g–i) daily total precipitation (PPT) and average volumetric soil moisture content (θ_v) in the upper (0–15 cm) and (0–30 cm) of soil, (j–l) mean green leaf area index (LAI) ± SE of the grazed and nongrazed (exclosure cages) areas and (m–o) dry aboveground biomass (AGB) of the grazed and nongrazed (exclosure cages) areas and dead material of the grazed area over the course of the study. DOY means day of year.

the warm-season C4 invasive species, *C. dactilon* L., remained green but with substantial differences in LAI between summers. The presence of this species during the summer is related to its drought tolerance (Mamolos *et al*., 2001; Vignolio *et al*., 2005). The length and area of its root system increase under water deficits (Vignolio *et al*., 2002), in order to enhance water absorption from deeper soil horizons, where moisture content

is higher. In 2005, the LAI between DOY 132 and 170 was above 0.1, but then decreased smoothly to approach zero towards the end of the summer (Fig. 1k). In 2006, the grass was mechanically cut on DOY 144, reducing slightly the LAI to 0.15 (Fig. 1l) and the aboveground biomass to 0.138 kg m^{-2} (Fig. 1o). The grass was left on the terrain covering partially the green leaves until DOY 162, when it was finally removed. The strong rain pulse just afterwards led to a quick development of the C4 grass, with LAI averaging 0.35 on DOY 188. After this, LAI remained almost constant until DOY 213. Thereafter, several grazing events reduced gradually its value to about 0.1, on DOY 217. An increase in LAI was observed again on DOY 270, because of the previous strong rain events that promoted the C3 grass seeds germination.

Seasonal and interannual variation in NEE, GPP and R_{eco}

Figure 2a–c shows the pattern of daily-integrated NEE, GPP and R_{eco} during the course of the study. The seasonal evolution of the cumulative values of NEE, GPP and R_{eco}, for the two hydrological years, is shown in Fig. 2d–f. After the first rains in autumn, the C3 grass seeds germinated and GPP was rapidly stimulated. The ecosystem became a daily carbon sink (negative NEE) in autumn, but around 30 days earlier in the autumn of 2005 than in 2004. This may be attributed to earlier grass seed germination (about 10 days) and slightly higher LAI in the autumn of 2005 than in 2004. In the winter of 2005, the ecosystem experienced a severe drought, especially in late winter when the average soil moisture of the top 15 cm of soil dropped below 10%. As a consequence, GPP and R_{eco} remained low and the daily NEE was close to zero. To the contrary, in the winter of 2006, GPP and R_{eco} increased gradually, but the increasing dominance of GPP over R_{eco} led the ecosystem to absorb gradually more carbon. In 2005, some early spring rain promoted a quick grass development, but a subsequent drought did not allow the ecosystem to reach a LAI as high as that observed in 2006. Hence, the carbon sequestration was limited and the maximum NEE (−2.4 g C m^{-2} day^{-1}) was only half of that observed in 2006 (−5.1 g C m^{-2} day^{-1}).

The 95th percentile values of the probability distribution of GPP and R_{eco} were 3.3 and 3.1 g C m^{-2} day^{-1}, respectively, for the first hydrological year, and 9.3 and 6.3 g C m^{-2} day^{-1}, for the second. We reported the 95th percentiles instead of the maximum values because the latter are less stable statistically. Regarding the dry year (2004–2005), the maximum NEE lies within the range of values reported for other water-stressed grasslands or dry years with similar LAI (Verhoef *et al.*, 1996; Flanagan *et al.*, 2002; Hunt *et al.*, 2002, 2004; Novick *et al.*, 2004; Li *et al.*, 2005). For the normal year (2005–2006), the maximum NEE and the 95th percentile values of GPP and R_{eco} were similar to those maximums (−4.8, 10.1, and 6.5 g C m^{-2} day^{-1}) reported for a Mediterranean C3 grassland in California (Xu & Baldocchi, 2004).

The end of the senescence of the C3 grasses occurred by about the DOY 132 in both hydrological years. In 2005, GPP varied from 2 g C m^{-2} day^{-1}, just after the

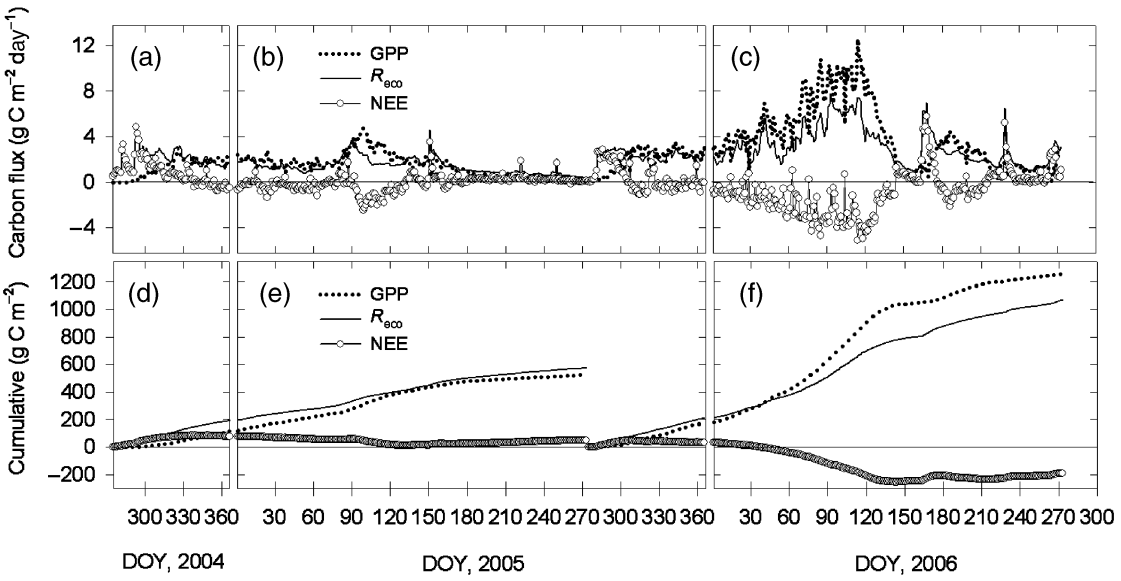


Fig. 2 Seasonal variation in daily-integrated (a–c) net ecosystem carbon exchange (NEE), gross primary production (GPP) and ecosystem respiration (R_{eco}) over the course of the study. Are also shown the cumulative NEE, GPP and R_{eco} for (d and e) the 2004–2005 and (e and f) the 2005–2006 hydrological years. Negative NEE means that the ecosystem is gaining carbon.

senescence, to 0.3 g C m^{-2} day^{-1} at the end of the summer, following the reductions in soil moisture content and LAI of the C4 grass (Fig. 2b). The GPP was consistently lower than R_{eco} and, hence, the daily NEE was slightly positive, as observed in a Mediterranean grassland with no vegetation during the summer (Xu & Baldocchi, 2004). This showed that with low LAI (around 0.1) the carbon fixed by the warm-season C4 grass did not compensate the larger carbon losses via plant and microbial respiration.

In 2006, the period after the end of the senescence of the C3 species offered a unique opportunity to understand how different management practices and the variability in soil moisture affected the direction and magnitude of NEE. Owing to the grass cutting, on DOY 144, the sign of NEE switched immediately from negative (NEE around -1 g C m^{-2} day^{-1}) to positive (NEE around 0.7 g C m^{-2} day^{-1}) (Fig. 2c). The same was observed, for example, in a warm temperate grassland after the harvest (Novick *et al*., 2004). However, in our case, the grass was left on the soil–plant surface, covering partially the remaining green leaves until DOY 162. Just afterwards, strong rain events increased the soil moisture and a large amount of carbon was immediately released by the ecosystem (Fig. 2c). The LAI of the C4 grass increased rapidly and 12 days later the ecosystem became a daily net carbon sink. This appears to be a common response of a water-stressed grassland whenever a rain event is strong enough to infiltrate and maintain water into the soil to a depth where it can be absorbed by roots, thus stimulating the plant growth (Huxman *et al*., 2004). In that period, the maximum NEE (-2.1 g C m^{-2} day^{-1}) was recorded on DOY 186 when LAI was reaching the maximum (0.35). After DOY 213, several grazing events reduced the LAI and hence the ecosystem became again a net source of carbon.

During the summer, we observed relevant carbon losses when the dry soil was rewetted by isolated rain events. Similar events were observed in Mediterranean ecosystem (Pereira *et al*., 2004; Xu & Baldocchi, 2004; Xu *et al*., 2004; Jarvis *et al*., 2007) and have been attributed to the so-called Birch effect, i.e., the quick activation of soil microbial respiration (Birch, 1958; Wu & Brookes, 2005) with the consequent mineralization of organic matter and nutrient release. Although we did not measure directly soil microbial respiration, because of the respiration and GPP of the C4 grass, the high positive values of NEE observed after the rain events must be explained by this effect as the C4 grass growth has to be necessarily a carbon sink.

We learnt from those previous studies on Mediterranean ecosystems that the short-term carbon losses, particularly after strong summer rain events, may exert negative effects on the annual NEE. For example, in a nearby site, Jarvis *et al*. (2007) demonstrated, using soil chambers, that a rain event of 18 mm resulted in a loss of 18 g C m^{-2} within the subsequent 10 days. In our study, however, the presence of the warm-season C4 grass during the summer played an important role, particularly by avoiding that strong rain events resulted in strong negative effects on the annual NEE. While small rain events (Fig. 3a) or even medium-size rain events, as observed on DOY 229 of 2006, resulted in carbon losses only (because the minor changes in soil moisture did not enhance the C4 grass growth), strong rain events as observed after DOY 163 of 2006 (Fig. 3b), led to subsequent growth of the C4 grass and consequent carbon sequestration. As a consequence, the carbon gain between DOY 177 and 213 (31 g C m^{-2}) compensated for most of the carbon loss between DOY 164 and 176 (38 g C m^{-2}).

To better understand the seasonal and interannual differences in NEE, GPP and R_{eco} we divided each hydrological year into three main periods of growth (Table 1), similar to those defined by Xu & Baldocchi (2004). Pregrowth was defined as the period between 1 October and 31 December. Growth was defined as the period between 1 January and the end of the C3 species senescence (12 May, DOY 132, in both hydrological years). The remaining period, until 30 September, was

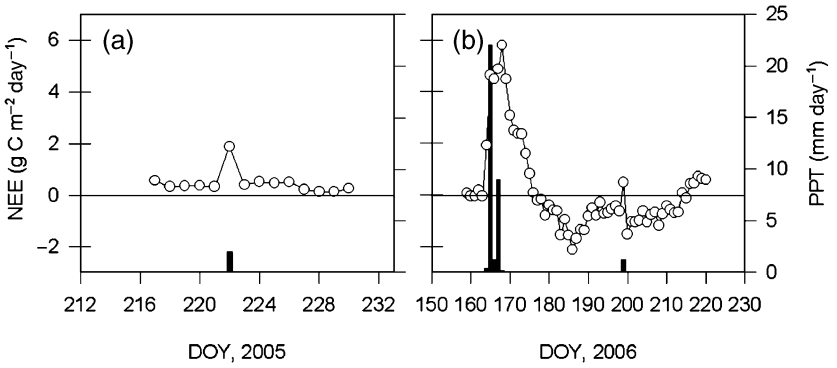


Fig. 3 Dynamic of NEE after (a) a small rain event and (b) strong rain events during the summer.

Table 1 Relationship between precipitation (PPT), average volumetric soil moisture content in the top 15 cm of soil (θ_v), net ecosystem carbon exchange (NEE), gross primary production (GPP) and ecosystem respiration (R_{eco}) at different periods of growth for the 2004–2005 and 2005–2006 hydrological years

Year	2004	2005	2005	2005	2006	2006	2004–2005	2005–2006
Period	Pregrowth	Growth	Dry	Pregrowth	Growth	Dry	Hydrological year	Hydrological year
PPT (mm)	239.4	83.2	41.0	355.2	290.2	105.8	363.6	751.2
θ_v (%)	24.1	10.8	5.3	26.0	31.1	10.1	10.9	21.8
GPP ($g C m^{-2}$)	115	289	120	177	815	269	524	1261
R_{eco} ($g C m^{-2}$)	193	221	159	212	536	323	573	1071
NEE ($g C m^{-2}$)	78	−68	39	35	−279	54	49	−190

defined as Dry, the time when only the warm-season C4 grass was present. A close inspection of Table 1 shows that the GPP was positively correlated with the total amount of precipitation, between the same periods of growth and the two hydrological years. Reductions in GPP were accompanied by reductions in R_{eco}, which is consistent with a number of observations during the European heatwave of 2003 (Ciais *et al*., 2005). This was also found by Reichstein *et al*. (2007) after analysing the data sets from numerous European eddy covariance flux sites. However, after comparing the two years, the lack of precipitation during the first hydrological year had more impact on GPP than on R_{eco}. Therefore, whereas in the normal year (2005–2006) the ecosystem was a net carbon sink ($-190\,g C m^{-2}$), in the dry year (2004–2005) the ecosystem was a net source of carbon to the atmosphere ($49\,g C m^{-2}$). For the 2 years of experiment, the total amount of precipitation was the main factor in determining the interannual variation in NEE, which supports previous findings (Flanagan *et al*., 2002; Suyker *et al*., 2003). In contrast, Xu & Baldocchi (2004) found that the timing of rain events had more impact on the NEE of a Mediterranean C3 grassland, by influencing both the length of the growing season and R_{eco}. Nevertheless, their study was conducted over two seasons (2000–2001 and 2001–2002) with similar amounts of precipitation and no soil moisture deficits during the growing seasons. Thus, because our ecosystem is similar to that Mediterranean C3 grassland, we may expect the timing of rain events to be the best predictor of carbon sequestration in years with similar total amounts of precipitation.

In the dry periods (Table 1), the ecosystem was a net carbon source to the atmosphere, even thought the carbon fixed by the warm-season C4 grass was around 23% and 21% of the total GPP of the first and second hydrological years, respectively. Nevertheless, as discussed above, the presence of this species played an important role due to its regrowth after the strong rain events in the late spring of 2006, causing a positive effect on the annual NEE. Another positive effect of its presence on the annual NEE was evident after the end of the senescence of the C3 grasses, in 2006, when the C4 grass was able to maintain the NEE at around $-1 g C m^{-2} day^{-1}$, for 12 days (Fig. 2c). After these 12 days, the grass cutting switched the NEE to positive values as previously discussed and, thereby, it was not possible to evaluate how long the ecosystem could remain as a carbon sink.

Seasonal and interannual variation in WUE_{GPP} and LUE_{GPP}

Figure 4a–c shows the seasonal variation in water-use efficiency (WUE_{GPP}) over the course of the study. Because of the large day-to-day variation in WUE_{GPP}, especially during wet periods, we calculated WUE for consecutive 5-day periods. WUE_{GPP} reached maximum values in early winter, specifically around 8 and $11\,mmol\,mol^{-1}$ in the first and second hydrological years, respectively. Towards the end of the summer, WUE_{GPP} declined to values of about $2\,mmol\,mol^{-1}$ in both hydrological years. Reductions in WUE_{GPP}, in response to drought, were previously reported for three Mediterranean evergreen ecosystems (Reichstein *et al*., 2002b). During the peak growth period of the first hydrological year, WUE_{GPP} was only half ($4\,mmol\,mol^{-1}$) of that measured in the second. For comparison, we may cite a study conducted on a northern temperate grassland during 3 years of contrasting rainfall (Wever *et al*., 2002). They report, for a 2-week period at the peak of the GPP in each year, lower daily average values of WUE_{GPP}, ranging from 2.52 to $3.13\,mmol\,mol^{-1}$ among years.

Ecological light-use efficiency (LUE_{GPP}) differed markedly between the two hydrological years (Fig. 4d–f). Previously, we showed that during the first

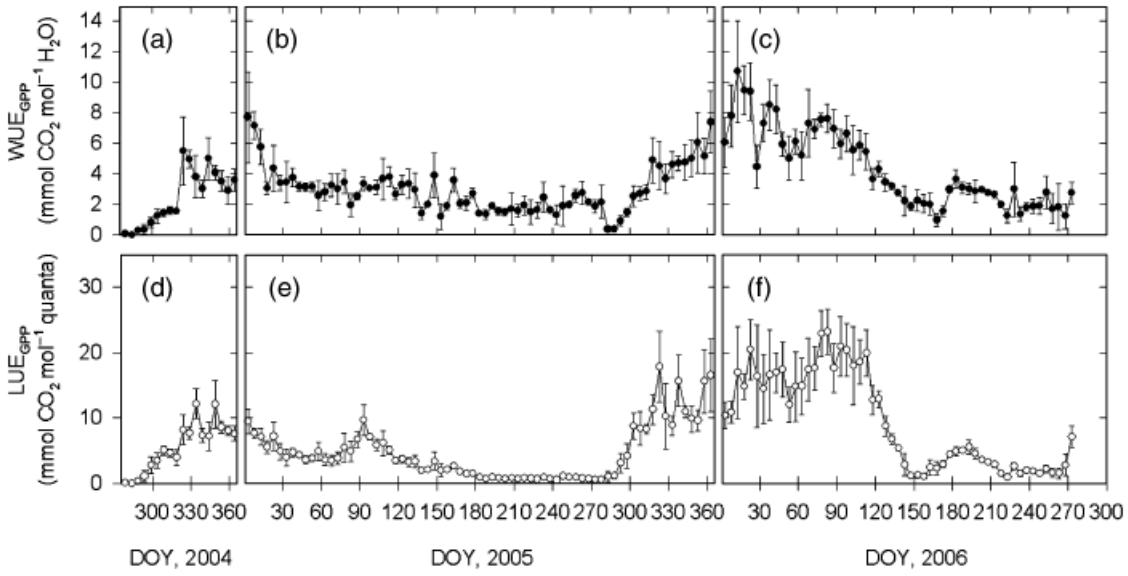


Fig. 4 Seasonal variation in (a–c) water-use efficiency (WUE_{GPP}) and (d–f) light-use efficiency (LUE_{GPP}) over the course of the study. Data represent 5-day averages (± SE).

and dry hydrological year the maximum LUE_{GPP} (10.9 mmol mol^{-1}, weekly average) of the Tojal grassland was similar to the values of other European semiarid grasslands (Gilmanov *et al*., 2007). In this study, the maximum 5-day average was fairly similar. For the second and normal hydrological year, the maximum LUE_{GPP} (measured near the maximum growth rate) was substantially higher (23 mmol mol^{-1}), thus approaching the values for temperate grasslands. The lowest LUE_{GPP} values were measured during the summer 0.5 and 0.8 mmol mol^{-1} in the first and second hydrological years, respectively.

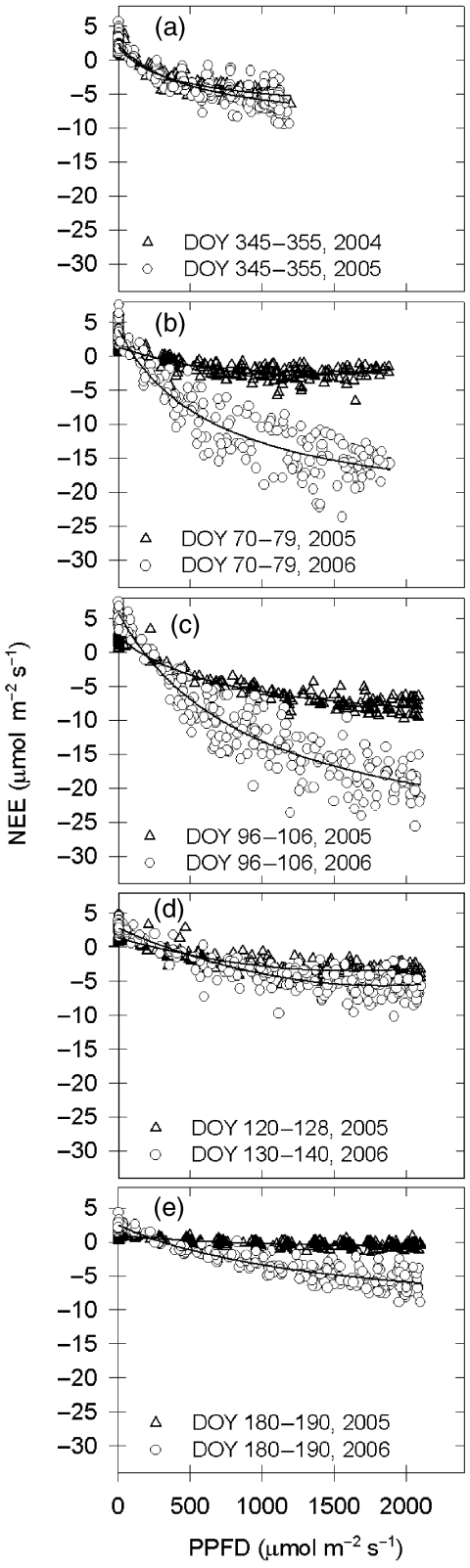
Controls on NEE, GPP and R_{eco}

NEE in response to PPFD. To assess the response of daytime NEE to incident PPFD, we present in Fig. 5 light–response curves for short periods of the main stages of plant growth of the two hydrological years. The main biophysical factors and the regression coefficients of the fitted curves, for the selected periods, are summarized in Table 2. For some periods, described in Fig. 5, light saturation of NEE was not found and, thus, a quadratic polynomial function was fitted to the data. In general, more than 90% of the variation in NEE was explained by the changes in PPFD (Table 2), which is consistent with results reported for a Mediterranean C3 grassland (Xu & Baldocchi, 2004).

The maximum values of NEE_{max} were −12.16 and −31.00 μmol m^{-2} s^{-1} for the first and second hydrological years, respectively, and were observed during the period of maximum LAI (DOY 96–106). The soil moisture and LAI were the main factors in determining this interannual variation. A large interannual variation was also observed by Flanagan *et al*. (2002) during 3 years of contrasting rainfall and LAI. In comparison, the maximum NEE_{max} value of the first hydrological year was similar with that (−9.60 μmol m^{-2} s^{-1}) reported for a semiarid steppe (Li *et al*., 2005). For the second hydrological year, the maximum NEE_{max} value was slightly lower than the value (−40.2 μmol m^{-2} s^{-1}) published for the Mediterranean C3 grassland (Xu & Baldocchi, 2004). The maximum quantum yield (α) also differed between hydrological years, specifically −0.024 and −0.040 for the first and second years, respectively. Although the latter was substantially higher than that observed by Xu & Baldocchi (2004), it was within the range of values for C3 and C4 grasslands (Ruimy *et al*., 1995).

In the late winter of 2005 (DOY 70–79), with soil water deficit, we observed a depression in NEE at the highest levels of PPFD (Fig. 5b). This depression has been widely observed in semiarid steppes (Li *et al*., 2005; Fu *et al*., 2006) and two effects concur to explain it. First, a reduction in photosynthesis due to the midday stomatal closure at high irradiance, temperature and vapour pressure deficit, when the leaf water potential is low (Jarvis & Morrison, 1981; Schulze & Hall, 1982). Second, enhanced ecosystem respiration at high temperatures (Xu & Baldocchi, 2004). However, after analysing our data, we concluded that the reduction in NEE was more due to a decrease in photosynthesis than an increase in ecosystem respiration as the temperature and vapour pressure deficit increased.

A relatively high value of NEE_{max} (−12.17 μmol $m^{-2} s^{-1}$) was observed in the summer of 2006 (DOY 180–190) due to the warm-season C4 grass regrowth (Fig. 5e). The α value (−0.001) was the lowest over the course of the study and was close to the values reported for a semiarid steppe (Li *et al*., 2005).



NEE and GPP in response to LAI. For the whole study period, GPP and NEE responded linearly to the changes in LAI (Fig. 6). The changes in LAI explained 77% and 84% of the variation found in NEE and GPP, respectively. The remaining percentage may be related to changes in environmental and soil variables. The strong linear relationship between NEE and LAI was also documented for a northern temperate grassland (Flanagan *et al*., 2002), but lower correlation was reported by Li *et al*. (2005) for a semiarid steppe. The results of the linear relationship between GPP and LAI agreed favourably with those ($r^2 = 0.84$, slope = 3.9) reported for a Mediterranean C3 grassland in California (Xu & Baldocchi, 2004). A similar slope (ca. 3.1) was also found for the semiarid steppe.

R_{eco} *in response to soil temperature and GPP.* Ecosystem respiration is a function of temperature. However, the temperature sensitivity of R_{eco} is affected by variations in soil moisture and substrate availability (Davidson *et al*., 2006). Because Mediterranean ecosystems show a large seasonal variation in soil water content and canopy growth, we determined the temperature sensitivity of R_{eco} for selected periods, where the LAI and soil moisture were similar (Fig. 7). The regression coefficients of the fitted curves, for the selected periods, are presented in Table 3. For the two hydrological years, the temperature sensitivity coefficient (Q_{10}) decreased from about 2.3, early in the growing season and with abundant soil moisture, to 1.22, during the dry summer. The same was found in a nearby evergreen oak savanna (Pereira *et al*., 2004) where the apparent Q_{10} of soil respiration, measured with a field chamber, decreased from more than 2 at 20% v/v soil moisture content to <1 in dry soil (cf. Jarvis *et al*., 2007). Similar reductions in Q_{10} related to decreases in soil moisture and increases in air temperature were documented for other Mediterranean ecosystems (Reichstein *et al*., 2002a; Xu & Baldocchi, 2004). In the Mediterranean C3 grassland, Xu & Baldocchi (2004) estimated a narrower variation in Q_{10} values, ranging from 2.51 to 2.11 during the season

Fig. 5 Seasonal and interannual variation in the light–response curves. Are shown different periods of growth of the 2004–2005 (triangle up) and 2005–2006 (circles) hydrological years. The curves were fitted using Eqn (3), except for the following periods where the quadratic polynomial function was used: DOY 70–79, 2005; DOY 120–128, 2005; DOY 180–190, 2005 and DOY 130–140, 2006. Regression coefficients are presented in Table 2.

Table 2 Leaf area index (LAI), average volumetric soil moisture content in the top 15 cm of soil (θ_v), air temperature (T) and regression coefficients as described in Eqn (3) for the selected periods in Fig. 5

Period	LAI	θ_v (%)	T (°C)	α	NEE_{max} ($\mu mol\,CO_2\,m^{-2}\,s^{-1}$)	R_{eco} ($\mu mol\,CO2\,m^{-2}\,s^{-1}$)	r^2
DOY 345–355, 2004	<1	23.6	9.1	−0.025	−7.52	1.92	0.95
DOY 345–355, 2005	~ 1	30.9	4.9	−0.024	−10.08	2.32	0.90
DOY 70–79, 2005	<1	7.8	13.6	–	–	–	–
DOY 70–79, 2006	~ 1.8	34.5	11.0	−0.040	−24.16	3.99	0.92
DOY 96–106, 2005	<1	8.7	12.5	−0.018	−12.16	2.42	0.95
DOY 96–106, 2006	~ 2.5	29.2	12.1	−0.039	−31.00	5.85	0.92
DOY 120–128, 2005	~ 0.3	5.7	17.2	–	–	–	–
DOY 130–140, 2006	~ 0.5	9.1	17.8	–	–	–	–
DOY 180–190, 2005	~ 0.1	5.1	23.2	–	–	–	–
DOY 180–190, 2006	~ 0.35	13.4	20.6	−0.001	−12.17	2.50	0.95

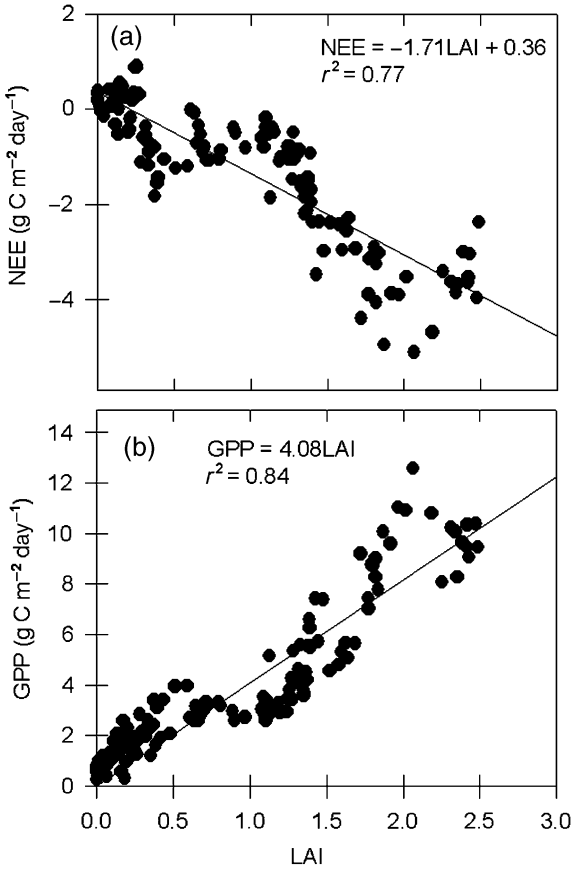


Fig. 6 The relationship between leaf area index (LAI) and the daily-integrated values of (a) net ecosystem carbon exchange (NEE) and (b) gross primary productivity (GPP) for the whole study period. It was used only data from cloudless or near-cloudless days.

2000–2001. Yet Flanagan & Johnson (2005) reported Q_{10} values varying from about 2.5 to 1.5 in the northern temperate grassland.

The daily R_{eco} values were strongly correlated with daily GPP values (Fig. 8). For the two growing seasons of the C3 grasses, GPP explained 85% of the variations in R_{eco}. Even during the summer of both hydrological years, the photosynthesis of the C4 grass strongly controlled R_{eco} ($r^2 > 0.73$). This shows that the canopy photosynthesis was the best indicator of R_{eco} by controlling the substrate availability for the autotrophic respiration and heterotrophic respiration through roots exudates (Davidson *et al.*, 2006). For comparison, Xu & Baldocchi (2004) also found a similar relationship ($r^2 = 0.78$) during the growing season. Strong influence of canopy photosynthesis on R_{eco} was documented for other grasslands ecosystems (Chimner & Welker, 2005; Li *et al.*, 2005) and forests (Janssens *et al.*, 2001). Other evidences come from experiments, such as large-scale tree-girdling (Högberg *et al.*, 2001) and manipulation of photosynthesis by clipping and shading grasses (Craine *et al.*, 1998; Wan & Luo, 2003), which demonstrated that soil respiration was closely related to canopy photosynthesis.

Effects of grazing events on NEE. Grazing occurred from late October to early February, in the first hydrological year, and from late October to late December, in the second, one or two times per week with a stocking density of 60 sheep ha^{-1}. Because grazing events reduce the LAI, they must be considered factors that control the NEE. We could only identify and follow in the filed few grazing events. Thus, as an example, in this section we only discuss the effect of a grazing event that occurred on DOY 349 of 2005. In Fig. 9 we show the impact of that grazing event on the diurnal pattern of NEE. The maximum NEE, which occurred by noon, was reduced from about 9 to 6 $\mu mol\,CO_2\,m^{-2}\,s^{-1}$, after the grazing event, as the result of LAI reduction. The

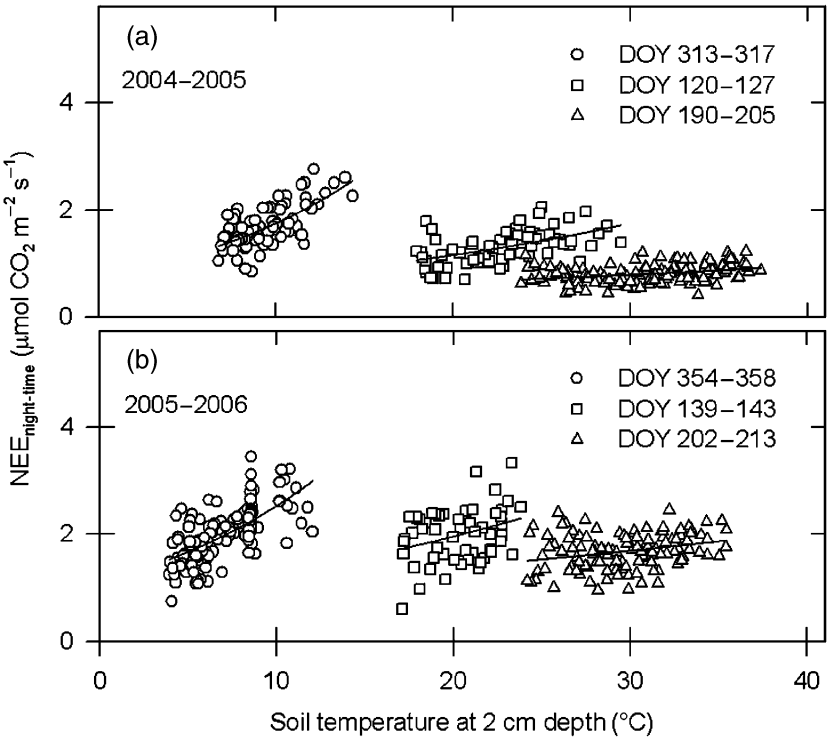


Fig. 7 The relationship between night-time half-hourly carbon dioxide flux density ($NEE_{night\text{-}time}$) and soil temperature at 2 cm depth for (a) the 2004–2005 and (b) the 2005–2006 hydrological years. Are shown different periods of growth, where the soil moisture and LAI were similar. The curves were fitted using Eqn (4), and the regression coefficients are presented in Table 3.

Table 3 Average volumetric soil moisture content in the top 15 cm of soil (θ_v) and regression coefficients as described in Eqn (4) for the selected periods in Fig. 7

Period	θ_v (%)	α	b	r^2	Q_{10}
DOY 313–317, 2004	27.1	0.74	0.086	0.45	2.36
DOY 120–127, 2005	5.7	0.50	0.042	0.28	1.52
DOY 190–205, 2005	4.7	0.43	0.020	0.10	1.22
DOY 354–358, 2005	29.3	1.11	0.083	0.44	2.29
DOY 139–143, 2006	8.4	0.84	0.043	0.10	1.54
DOY 202–213, 2006	7.7	0.92	0.020	0.08	1.22

irregular pattern on DOY 349 shows the influence of sheep respiration.

The impact of the grazing event on the light–response curves is shown in Fig. 10. We analysed a period of 10 days around the grazing event (5 days before and after), in which the wind sector and the major meteorological parameters were similar. The NEE_{max} was substantially reduced from −13.7 to $-8.9\,\mu mol\,CO_2\,m^{-2}\,s^{-1}$, after the grazing event. The daily NEE was also affected; the mean daily NEE for the 5-day period before and after the grazing event was −0.49 and $-0.11\,g\,C\,m^{-2}$, respectively. However, it is worth mentioning that these mean values may be affected by the uncertainties underlying the gap-filling of the missing nocturnal periods.

Although grazing events exert a short-term negative impact on daily NEE, the impact of grazing on NEE relatively to a nongrazed area is unclear. As previously discussed, we observed a negative effect of grazing on LAI, mainly during the winter. However, the impact on NEE relative to a nongrazed area depends on how the grazing affects the processes that control R_{eco} and GPP. For this reason, the impact of grazing seems to vary among studies. For example, Rogiers *et al*. (2005) reported a negative effect of cattle grazing on the NEE of a grassland in the Swiss Alps. Other studies have found no significant difference in NEE between grazed and nongrazed areas (LeCain *et al*., 2002; Wilsey *et al*., 2002; Risch & Frank, 2006). Therefore, because understanding the effects of grazing on the annual NEE is crucial to develop a grazing management plan capable of avoiding strong negative effects or even improving the carbon uptake, further studies on this area are needed.

Conclusions

This study was conducted during two climatologically contrasting hydrological years (one dry 2004–2005, and

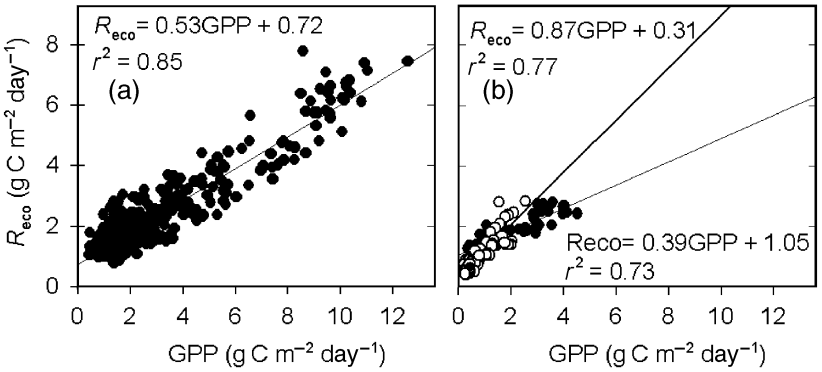


Fig. 8 The relationship between the daily-integrated values of ecosystem respiration (R_{eco}) and gross primary productivity (GPP). (a) Data from the growing seasons of the C3 species; (b) data from the summer of 2005 (white circles) and the summer of 2006 (black circles), when only the C4 grass was present. Rainy days were excluded from the analysis.

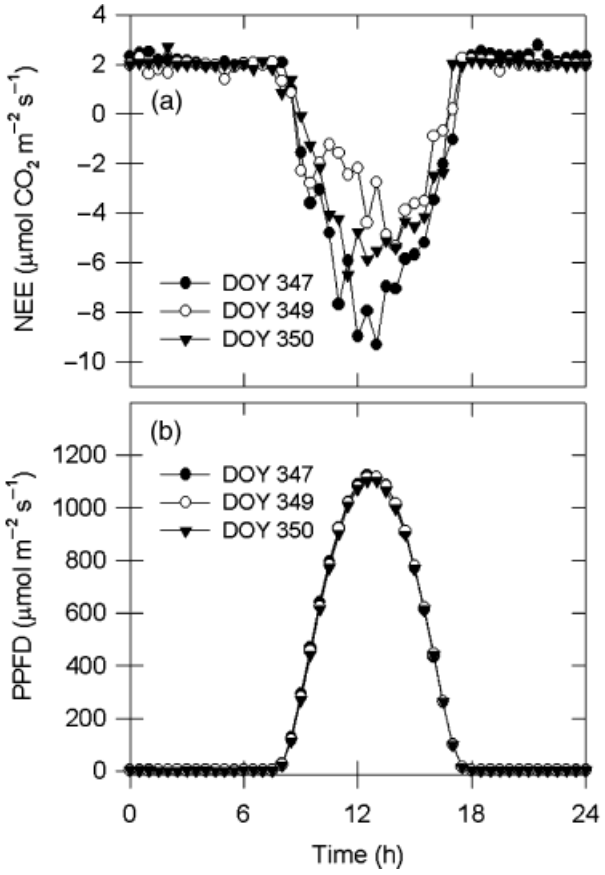


Fig. 9 The effects of grazing on the diurnal pattern of net ecosystem carbon dioxide exchange (NEE). Are shown a day before (DOY 347, 2005) and the day after (DOY 350, 2005) of the grazing event on DOY 349 of 2005. The PPFD was similar as well as the wind sector. In (a) most of the night-time periods were gap filled.

another normal 2005–2006) and, thereby, offered a unique opportunity to understand how interannual climate variability affects the CO_2 exchange between a Mediterranean grassland and the atmosphere. In the dry year, grass production and LAI were severely affected by the soil moisture deficits during the winter and early spring. As a consequence, the maximum daily rate of NEE was approximately half of that measured in the normal year. Similarly, during the peak growth period of the normal year, light-use and water-use efficiencies were twice as high as those observed in the dry year. After the end of the senescence of the C3 grasses, the carbon fixed by the warm-season C4 grass was around 23% and 21% of the total GPP of the dry and normal years, respectively. Nevertheless, the ecosystem was a carbon source in those periods. However, particularly in the normal year, the presence of this C4 grass played an important role with a positive impact on the annual NEE, essentially by converting the ecosystem into a carbon sink after strong rain events and extending the ecosystem carbon sequestration for several days, after the end of the senescence of the C3 grasses. Thus, the presence of this warm-season C4 grass, and particularly its response to rain events, should be taken into consideration when modelling the NEE, GPP and R_{eco} of Mediterranean grasslands with some contingent of C4 species.

For the 2 years of the experiment, the total amount of precipitation was the main factor in determining the interannual variation in NEE. Hence, this grassland lost 49 g C m^{-2} to the atmosphere during the dry year and captured 190 g C m^{-2} during the normal year. For the whole study period, the ecosystem was even a considerable sink for carbon (−141 g C m^{-2}), suggesting that this grassland has potential to sequester carbon. However, giving that we observed 2 years of contrasting amount of precipitation, much more years of study are needed to evaluate, for example, the influence of the timing of rain events on the annual NEE.

For this grassland, NEE and GPP were strongly related to the PPFD, on short-term time scales, and

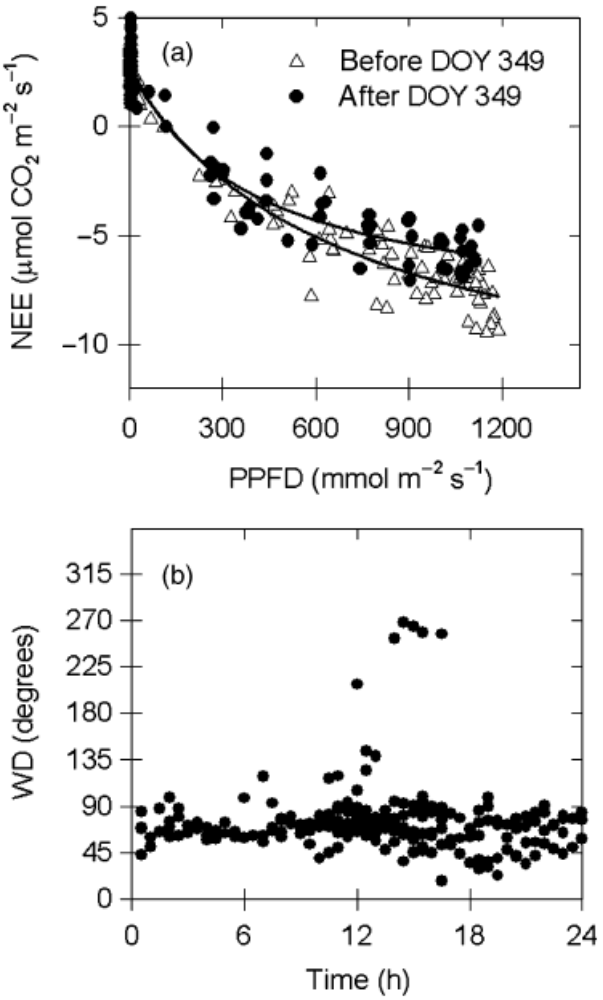


Fig. 10 The effects of grazing on the light–response curves. (a) Data represent 5 days before and 5 days after the grazing event on DOY 349 of 2005. The curves were fitted using Eqn (3). (b) It is shown the wind direction (WD) during the whole period of analysis.

LAI, on long-term time scales. The variations in R_{eco} were mainly controlled by the canopy photosynthesis. The grazing events during the autumn of the normal year limited the development of LAI and aboveground biomass. The impact was large during the winter, but reduced during the peak growth period. After each grazing event, the reduction in LAI affected negatively the NEE. However, because it is imperative to define grazing management plans, further studies are needed to evaluate the impact of grazing on the annual NEE, relatively to a nongrazed area.

Acknowledgements

The authors thank 'Fundação para a Ciência e a Tecnologia' for financial support through a PhD grant to L. M. I. Aires. This work was performed under the CARBOEUROPE-IP European Project financed by the European Community.

References

Anthoni PM, Freibauer A, Kolle O, Schulze ED (2004) Winter wheat carbon exchange in Thuringia, Germany. *Agricultural and Forest Meteorology*, **121**, 55–67.

Birch HF (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Plant Soil*, **10**, 9–31.

Chimner RA, Welker JM (2005) Ecosystem respiration responses to experimental manipulations of winter and summer precipitation in a Mixedgrass Prairie, WY, USA. *Biogeochemistry*, **73**, 257–270.

Chocarro C, Lloveras J, Fanlo R (2005) Effects of winter grazing on spring production of lucerne under Mediterranean conditions. *Grass and Forage Science*, **60**, 146–150.

Ciais P, Reichstein M, Viovy N *et al*. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529–533.

Craine JM, Wedin DA, Chapin FS (1998) Predominance of ecophysiological controls on soil CO2 flux in a Minnesota grassland. *Plant and Soil*, **207**, 77–86.

Davidson EA, Janssens IA, Luo YQ (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q(10). *Global Change Biology*, **12**, 154–164.

Falge E, Baldocchi D, Olson R *et al*. (2001) Gap filling strategies for defensible annual sums of net ecosystem exchange. *Agricultural and Forest Meteorology*, **107**, 43–69.

Figueroa ME, Davy AJ (1991) Response of Mediterranean grassland species to changing rainfall. *Journal of Ecology*, **79**, 925–941.

Flanagan LB, Johnson BE (2005) Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agricultural and Forest Meteorology*, **130**, 237–253.

Flanagan LB, Wever LA, Carlson PJ (2002) Seasonal and interannual variation in carbon dioxide exchange and carbon balance in a northern temperate grassland. *Global Change Biology*, **8**, 599–615.

Foken T, Wichura B (1996) Tools for quality assessment of surface-based flux measurements. *Agricultural and Forest Meteorology*, **78**, 83–105.

Fu YL, Yu GR, Sun XM *et al*. (2006) Depression of net ecosystem CO2 exchange in semi-arid Leymus chinensis steppe and alpine shrub. *Agricultural and Forest Meteorology*, **137**, 234–244.

Fuehrer PL, Friehe CA (2002) Flux corrections revisited. *Boundary-Layer Meteorology*, **102**, 415–457.

Gilmanov TG, Soussana JF, Aires L *et al*. (2007) Partitioning European grassland net ecosystem CO_2 exchange into gross primary productivity and ecosystem respiration using light response function analysis. *Agriculture Ecosystems and Environment*, **121**, 93–120.

Gockede M, Markkanen T, Hasager CB, Foken T (2006) Update of a footprint-based approach for the characterisation of complex measurement sites. *Boundary-Layer Meteorology*, **118**, 635–655.

Högberg P, Nordgren A, Buchmann N *et al*. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.

Hollinger DY, Goltz SM, Davidson EA, Lee JT, Tu K, Valentine HT (1999) Seasonal patterns and environmental control of carbon dioxide and water vapour exchange in an ecotonal boreal forest. *Global Change Biology*, **5**, 891–902.

House JI, Hall DO (2001) Productivity of tropical Savannas and grasslands. In: *Terrestrial Global Productivity* (eds Roy J, Saugier B, Mooney HA), pp. 363–400. Academic Press, San Diego.

Hunt JE, Kelliher FM, McSeveny TM, Byers JN (2002) Evaporation and carbon dioxide exchange between the atmosphere and a tussock grassland during a summer drought. *Agricultural and Forest Meteorology*, **111**, 65–82.

Hunt JE, Kelliher FM, McSeveny TM, Ross DJ, Whitehead D (2004) Long-term carbon exchange in a sparse, seasonally dry tussock grassland. *Global Change Biology*, **10**, 1785–1800.

Huxman TE, Snyder KA, Tissue D *et al*. (2004) Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia*, **141**, 254–268.

INMG (1991) O Clima de Portugal. Normais climatológicas da região de Alentejo e Algarve, correspondentes a 1951–1980, Fascículo XLIX, Vol. 4–4a região. Instituto Nacional de Meteorologia e Geofísica, Lisboa, Portugal.

Janssens IA, Lankreijer H, Matteucci G *et al*. (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.

Jarvis PG, Morrison JIL (1981) The control of transpiration and photosynthesis by the stomata. In: *Stomatal Physiology* (eds Jarvis PG, Mansfield TA), pp. 247–279. Cambridge University Press, Cambridge.

Jarvis PG, Rey A, Petsikos C *et al*. (2007) Drying and wetting of soils stimulates decomposition and carbon dioxide emission: the ''Birch Effect''. *Tree Physiology*, **27**, 929–940.

Kato T, Tang YH, Gu S *et al*. (2004) Carbon dioxide exchange between the atmosphere and an alpine meadow ecosystem on the Qinghai-Tibetan Plateau, China. *Agricultural and Forest Meteorology*, **124**, 121–134.

Kowalski S, Sartore M, Burlett R, Berbigier P, Loustau D (2003) The annual carbon budget of a French pine forest (*Pinus pinaster*) following harvest. *Global Change Biology*, **9**, 1051–1065.

LeCain DR, Morgan JA, Schuman GE, Reeder JD, Hart RH (2002) Carbon exchange and species composition of grazed pastures and exclosures in the shortgrass steppe of Colorado. *Agriculture Ecosystems and Environment*, **93**, 421–435.

Li SG, Asanuma J, Eugster W *et al*. (2005) Net ecosystem carbon dioxide exchange over grazed steppe in central Mongolia. *Global Change Biology*, **11**, 1941–1955.

Li SG, Eugster W, Asanuma J *et al*. (2006) Energy partitioning and its biophysical controls above a grazing steppe in central Mongolia. *Agricultural and Forest Meteorology*, **137**, 89–106.

Mamolos AP, Veresoglou DS, Noitsakis V, Gerakis A (2001) Differential drought tolerance of five coexisting plant species in Mediterranean lowland grasslands. *Journal of Arid Environments*, **49**, 329–341.

Meyers TP (2001) A comparison of summertime water and CO_2 fluxes over rangeland for well watered and drought conditions. *Agricultural and Forest Meteorology*, **106**, 205–214.

Miranda PMA, Coelho FES, Tomé AR *et al*. (2002) 20th century Portuguese Climate and Climate Scenarios. In: *Climate Change in Portugal: Scenarios, Impacts and Adaptation Measures (SIAM Project)* (eds Santos FD, Forbes K, Moita R), pp. 23–83. Gradiva, Lisboa.

Nagy Z, Pintér K, Czóbel S *et al*. (2007) The carbon budget of semi-arid grassland in a wet and a dry year in Hungary. *Agriculture Ecosystems and Environment*, **121**, 21–29.

Novick KA, Stoy PC, Katul GG *et al*. (2004) Carbon dioxide and water vapor exchange in a warm temperate grassland. *Oecologia*, **138**, 259–274.

Pereira JS, David JS, David TS, Caldeira MC, Chaves MM (2004) Carbon and water fluxes in Mediterranean-type ecosystems – constraints and adaptations. In: *Progress in Botany* (eds Esser K, Lüttge U, Beyschlag W, Murata J), pp. 467S–498S. Springer-Verlag, Berlin.

Rannik U, Markkanen T, Raittila J, Hari P, Vesala T (2003) Turbulence statistics inside and over forest: influence on footprint prediction. *Boundary-Layer Meteorology*, **109**, 163–189.

Reichstein M, Falge E, Baldocchi D *et al*. (2005) On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. *Global Change Biology*, **11**, 1424–1439.

Reichstein M, Papale D, Valentini R *et al*. (2007) Determinants of terrestrial ecosystem carbon balance inferred from European eddy covariance flux sites. *Geophysical Research Letters*, **34**, L01402, doi: 01410.01029/02006GL027880.

Reichstein M, Tenhunen JD, Roupsard O *et al*. (2002a) Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. *Functional Ecology*, **16**, 27–39.

Reichstein M, Tenhunen JD, Roupsard O *et al*. (2002b) Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? *Global Change Biology*, **8**, 999–1017.

Risch AC, Frank DA (2006) Carbon dioxide fluxes in a spatially and temporally heterogeneous temperate grassland. *Oecologia*, **147**, 291–302.

Rogiers N, Eugster W, Furger M, Siegwolf R (2005) Effect of land management on ecosystem carbon fluxes at a subalpine grassland site in the Swiss Alps. *Theoretical and Applied Climatology*, **80**, 187–203.

Ruimy A, Jarvis PG, Baldocchi DD, Saugier B (1995) CO_2 fluxes over plant canopies and solar radiation: a review. *Advances in Ecological Research*, **26**, 1–68.

Schulze ED, Hall AE (1982) Stomatal responses, water loss and CO_2 carbon dioxide assimilation rates of plants in contrasting environments. In: *Encyclopedia of Plant Physiology – Physiological Plant Ecology* (eds Lange OL, Nobel PS, Osmond CB, Ziegler H), pp. 181–230. Springer-Verlag, Berlin.

Schwinning S, Ehleringer JR (2001) Water use trade-offs and optimal adaptations to pulse-driven arid ecosystems. *Journal of Ecology*, **89**, 464–480.

Scott RL, Edwards EA, Shuttleworth WJ, Huxman TE, Watts C, Goodrich DC (2004) Interannual and seasonal variation in

fluxes of water and carbon dioxide from a riparian woodland ecosystem. *Agricultural and Forest Meteorology*, **122**, 65–84.

Sims PL, Bradford JA (2001) Carbon dioxide fluxes in a southern plains prairie. *Agricultural and Forest Meteorology*, **109**, 117–134.

Suyker AE, Verma SB, Burba GG (2003) Interannual variability in net CO_2 exchange of a native tallgrass prairie. *Global Change Biology*, **9**, 255–265.

Twine TE, Kustas WP, Norman JM *et al*. (2000) Correcting eddy-covariance flux underestimates over a grassland. *Agricultural and Forest Meteorology*, **103**, 279–300.

Valentini R, Gamon JA, Field CB (1995) Ecosystem gas-exchange in a California Grassland – Seasonal patterns and implications for scaling. *Ecology*, **76**, 1940–1952.

Verhoef A, Allen SJ, DeBruin HAR, Jacobs CMJ, Heusinkveld BG (1996) Fluxes of carbon dioxide and water vapour from a Sahelian savanna. *Agricultural and Forest Meteorology*, **80**, 231–248.

Vickers D, Mahrt L (1997) Quality control and flux sampling problems for tower and aircraft data. *Journal of Atmospheric and Oceanic Technology*, **14**, 512–526.

Vignolio OR, Biel C, de Herralde F, Araujo-Alves JPL, Save R (2002) Growth of *Lotus creticus creticus* and *Cynodon dactylon* under two levels of irrigation. *Australian Journal of Agricultural Research*, **53**, 1375–1381.

Vignolio OR, Biel C, de Herralde F, Araujo-Alves JPL, Save R (2005) Use of water-stress tolerant *Lotus creticus* and *Cynodon dactylon* in soil revegetation on different slopes in a Mediterranean climate. *Annales Botanici Fennici*, **42**, 195–205.

Wan SQ, Luo YQ (2003) Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment. *Global Biogeochemical Cycles*, **17**, 1054, doi: 1010.1029/2002GB01971.

Webb EK, Pearman GI, Leuning R (1980) Correction of flux measurements for density effects due to heat and water-vapor transfer. *Quarterly Journal of the Royal Meteorological Society*, **106**, 85–100.

Wever LA, Flanagan LB, Carlson PJ (2002) Seasonal and interannual variation in evapotranspiration, energy balance and surface conductance in a northern temperate grassland. *Agricultural and Forest Meteorology*, **112**, 31–49.

Wilsey BJ, Parent G, Roulet NT, Moore TR, Potvin C (2002) Tropical pasture carbon cycling: relationships between C source/sink strength, above-ground biomass and grazing. *Ecology Letters*, **5**, 367–376.

Wilson K, Goldstein A, Falge E *et al*. (2002) Energy balance closure at FLUXNET sites. *Agricultural and Forest Meteorology*, **113**, 223–243.

Wohlfahrt G, Anfang C, Bahn M *et al*. (2005) Quantifying nighttime ecosystem respiration of a meadow using eddy covariance, chambers and modelling. *Agricultural and Forest Meteorology*, **128**, 141–162.

Wu J, Brookes PC (2005) The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. *Soil Biology and Biochemistry*, **37**, 507–515.

Xu LK, Baldocchi DD (2004) Seasonal variation in carbon dioxide exchange over a Mediterranean annual grassland in California. *Agricultural and Forest Meteorology*, **123**, 79–96.

Xu LK, Baldocchi DD, Tang JW (2004) How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Global Biogeochemical Cycles*, **18**, GB4002.

